Appl. No. 10/056,662

Amendment to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (currently amended) A method for isolating islets from a portion of a pancreas, comprising:

introducing the portion of a pancreas to an islet processing solution that contains a digestive enzyme and that is characterized by a plurality of process control variables;

circulating said islet processing solution around and through the portion of a pancreas and past a plurality of sensors, said plurality of sensors being exposed to said islet processing solution having an output that characterizes a state of one of the process control variables;

controlling said plurality of process control variables of the islet processing solution during islet isolation with a process controller that is in communication with said plurality of sensors, said process controller having a process control interface and being capable of changing the state of said plurality of process control variables, wherein said plurality of process control variables include a temperature, a flowrate, a pH, a dissolved oxygen concentration, a dissolved nitric oxide concentration, an antibiotic concentration and an endotoxin concentration;

separating the islets from the portion of pancreas; and

collecting the separated islets.

2. (previously presented) The method of claim 1, wherein said controlling step comprises controlling said plurality of process control variables with a proportional, integral, derivative controller.

3. (previously presented) The method of claim 1, wherein said controlling step comprises controlling said plurality of process control variables with a microprocessor temperature controller.

4. (previously presented) The method of claim 1, wherein said controlling step comprises controlling said plurality of process control variables with is a microprocessor controller.

5. (previously presented) The method of claim 1, wherein said controlling step comprises controlling said plurality of process control variables with a microprocessor computer.

6. (previously presented) The method of claim 1, wherein said controlling step comprises controlling said plurality of process control variables with a variable resistance transformer.

7. (previously presented) The method of claim 1, wherein the temperature is adjusted by an electrical resistance element in thermal communication with the islet processing solution.

8. (previously presented) The method of claim 1, wherein the temperature is adjusted by steam placed in thermal communication with the islet processing solution.

9. (previously presented) The method of claim 1, wherein the temperature is adjusted by a recirculating fluid bath in thermal communication with the islet processing solution.

10. (previously presented) The method of claim 1, wherein the temperature is adjusted by the ambient temperature of the environment in thermal communication with the islet processing solution.

11. (previously presented) The method of claim 1, wherein the pH is controlled by a microprocessor pH controller between pH 6.00 and pH 8.00.

12. (previously presented) The method of claim 1, wherein the pH is controlled by a microprocessor controller between pH 6.00 and pH 8.00.

13. (previously presented) The method of claim 1, wherein the pH is controlled by a microprocessor computer between pH 6.00 and pH 8.00.

14. (previously presented) The method of claim 1, wherein the pH is controlled by the addition of an acid or base to the islet processing solution.

15. (cancelled)

16. (previously presented) The method of claim 1, wherein the flowrate is controlled by a microprocessor controller between 10.0 milliliters per minute and 4000.0 milliliters per.

17. (cancelled)

18. (cancelled)

19. (previously presented) The method of claim 1, wherein the dissolved oxygen concentration is controlled by a microprocessor controller between 0.000000001 milligrams per milliliter and 10.0 milligrams per milliliter.

20. (cancelled)

21. (previously presented) The method of claim 1, wherein the dissolved oxygen concentration is controlled by sparging the islet processing solution with at least one inert gas selected from the group consisting of helium, neon, argon, krypton and xenon.

22. (cancelled)

23. (previously presented) The method of claim 1, wherein the dissolved nitric oxide concentration is controlled by a microprocessor controller between 0.00000000000001 moles per liter and 1.0 mole per liter.

24. (cancelled)

25. (previously presented) The method of claim 1, wherein the dissolved nitric oxide concentration is controlled by sparging the islet processing solution with at least one inert gas selected from the group consisting of helium, neon, argon, krypton and xenon.

26. (cancelled)

27. (previously presented) The method of claim 1, wherein the endotoxin concentration is controlled by a microprocessor controller between 0.000000001 endotoxin units per milligram and 100.0 endotoxin units per milligram.

28. (cancelled)

29. (previously presented) The method of claim 1, wherein the endotoxin concentration is controlled by the addition of an endotoxin neutralizing protein to the islet processing solution.

30. (previously presented) The method of claim 29 wherein the endotoxin neutralizing protein concentration is controlled by a microprocessor controller between 0.00000000000001 moles per liter and 1.0 moles per liter.

31. (previously presented) The method of claim 1, wherein said plurality of process control the variables further comprise a digestive enzyme activity which is controlled by the addition of one or more antibiotics to the islet processing solution selected from the group consisting of tetracycline, minocycline and doxycycline.

32. (previously presented) The method of claim 1, wherein said plurality of process control the variables further comprise a digestive enzyme activity which is controlled by the

addition of one or more chelators of divalent cations to the islet processing solution selected from the group consisting of citrate, EDTA and EGTA.

33. (previously presented) The method of claim 1, wherein said plurality of process control the variables further comprise a digestive enzyme activity which is controlled by the addition of one or more amino acids to the islet processing solution selected from the group consisting of cysteine and cystine.

34. (previously presented) The method of claim 1, wherein said plurality of process control variables further comprise a digestive enzyme activity which is controlled by a microprocessor controller between 0.00000000000001 moles per liter and 1.0 moles per liter.

35. (cancelled)

36. (currently amended) The method of claim 1, wherein the antibiotic concentration is controlled by a microprocessor controller between 0.00000000000001 moles per liter A and 1.0 mole per liter A.

37. (previously presented) The method of claim 1, wherein said plurality of process control variables further comprise nitric oxide synthase activity which is controlled by the addition to the islet processing solution of one or more derivatives of L-arginine selected from

the group consisting of aminoguanidinie, N, N'-diaminoguanidine, methylguanidine and 1, 1-dimethylguanidine.

38. (previously presented) The method of claim 1, wherein the dissolved nitric oxide concentration is controlled by the addition of 2,4-diamino-6-hydroxy-pyrimidine to the islet processing solution.

39. (previously presented) The method of claim 1, wherein said plurality of process control variables further comprise a pressure which is between 1.0 pound per square inch gauge pressure and 150.0 pounds per square inch gauge pressure.

40. (previously presented) The method of claim 1, wherein said plurality of process control variables comprise a carbon monoxide concentration which is controlled by sparging the islet processing solution with carbon monoxide.

41. (previously presented) The method of claim 1, wherein the pancreas is a human pancreas.

42. (previously presented) The method of claim 1, wherein the pancreas is a transgenic porcine pancreas.

43. (previously presented) The method of claim 1, wherein the pancreas is a non-transgenic porcine pancreas.

44. (previously presented) The method of claim 1, wherein the pancreas is a transgenic mammalian pancreas.

45. (previously presented) The method of claim 1, wherein the pancreas is a non-transgenic mammalian pancreas.

46. (previously presented) The method of claim 1, wherein the pancreas is a transgenic fish pancreas.

47-60. (canceled)

61. (currently amended) A method for isolating islets from a pancreatic tissue, comprising:

a step for introducing the pancreatic tissue to an islet processing solution that contains a digestive enzyme and that is characterized by a plurality of process control variables;

a step for circulating said islet processing solution through the pancreatic tissue;

a step for controlling said plurality of process control variables of the islet processing solution during islet isolation, the plurality of process control variables comprising: a temperature, a pH, a flowrate, a dissolved oxygen concentration, a dissolved nitric oxide concentration, a nitric oxide synthase activity, an endotoxin concentration, an endotoxin neutralizing protein concentration, an antibiotic concentration, an amino acid concentration, a dextran concentration, a heparin concentration, and a digestive enzyme activity;

a step for separating the islets from the pancreatic tissue while the process control variables are controlled; and

a step for collecting the separated islets.

62. (previously presented) The method claim 61, wherein the digestive enzyme activity is controlled by adding an antibiotic to the islet processing solution.

63. (previously presented) The method claim 61, wherein the digestive enzyme activity is controlled by adding a chelator of divalent cations to the islet processing solution.

64. (previously presented) The method of claim 61, wherein the digestive enzyme activity is controlled by adding an amino acid to the islet processing solution.

65. (previously presented) The method of claim 61, wherein said dissolved nitric oxide concentration is controlled or inhibited by adding to the islet processing solution of one or more derivatives of L-arginine selected from the group consisting of aminoguanidinie, N, N'-diaminoguanidine, methylguanidine, and 1, 1-dimethylguanidine.

66. (previously presented) The method of claim 61, wherein said dissolved nitric oxide concentration is controlled or inhibited by adding 2,4-diamino-6-hydroxy-pyrimidine to the islet processing solution.

67. (previously presented) The method of claim 61, wherein said dissolved nitric oxide concentration is controlled or inhibited by adding an amino acid to the islet processing solution.

68. (previously presented) The method of claim 61, wherein said dissolved nitric oxide concentration is controlled or inhibited by adding to the islet processing solution one or more of the compounds selected from the group consisting of dextran and heparin.

69. (previously presented) The method of claim 61, wherein said dissolved nitric oxide concentration is controlled or inhibited by adding to the islet processing solution one or more antibiotics selected from the group consisting of tetracycline, minocycline, and doxycycline.

70. (previously presented) The method of claim 61, wherein the nitric oxide synthase activity is controlled or inhibited by adding to the islet processing solution one or more antibiotics selected from the group consisting of tetracycline, minocycline, and doxycycline.

71. (cancelled)

72. (cancelled)

73. (previously presented) The method of claim 61 wherein:

said step for controlling one or more of said plurality of process control variables is accomplished with a process controller.

74. (previously presented) The method of claim 61 wherein:

one or more of said plurality of process control variables is controlled in said step for controlling.

75. (previously presented) The method of claim 61 wherein:

one or more of said plurality of process control variables is controlled with a process controller in said step for controlling.

76. (withdrawn and currently amended) An apparatus for isolating islets from a pancreatic tissue, said apparatus comprising:

a heated feed tank;

~~an~~ a reactor for containing a physiologic process solution having a dissolved oxygen concentration, a dissolved nitric oxide concentration, a pH, a temperature, a pressure, an endotoxin concentration and a dissolved carbon dioxide concentration, said reactor comprising a flow loop that connects a digestion chamber, a sparging vessel and a process pump, said flow

loop being connected through a first valve to said heated feed tank, said process pump being operative to circulate said physiologic process solution through said reactor;

a process controller;

a sensor block that is located on said flow loop upstream from said digestion chamber, said sensor block having a plurality of sensors that send signals to said process controller, said sensor block comprising a dissolved oxygen sensor for sensing said dissolved oxygen concentration, a dissolved nitric oxide sensor for sensing said dissolved nitric oxide concentration, a pH sensor for sensing said pH, a temperature sensor for sensing said temperature, a pressure sensor for sensing said pressure, an endotoxin sensor assembly for sensing said endotoxin concentration, and a carbon dioxide sensor for sensing said dissolved carbon dioxide concentration;

a process cooler that is located on said flow loop and a process heater that is located on said flow loop;

a plurality of solution pumps comprising a digestive enzyme solution pump for pumping a digestive enzyme solution from a digestive enzyme solution reservoir into said flow loop, an endotoxin neutralizing protein solution pump for pumping an endotoxin neutralizing protein solution from an endotoxin neutralizing protein solution reservoir into said flow loop, an acid solution pump for pumping an acid solution from an acid solution reservoir into said flow loop, and a base solution pump for pumping a base solution from a base solution reservoir into said flow loop;

a source of oxygen gas controlled by a oxygen gas valve for introducing a stream of oxygen gas into said sparging vessel, a source of carbon dioxide gas controlled by a carbon dioxide gas valve for introducing a stream of carbon dioxide gas into said sparging vessel, and a

source of an inert gas controlled by an inert gas valve for introducing a stream of inert gas into said sparging vessel; and

an auto-collector that is connected to said flow loop for collecting the islets;

wherein said process controller is operative to compare the dissolved oxygen concentration in said physiologic process solution to a dissolved oxygen setpoint and activate either said oxygen gas valve to add dissolved oxygen or said inert gas valve to remove dissolved oxygen, to compare said dissolved carbon dioxide concentration to a dissolved carbon dioxide setpoint and activate said inert gas valve to remove dissolved carbon oxide, to compare said dissolved nitric oxide concentration to a dissolved nitric oxide setpoint and activate said inert gas valve to remove dissolved oxygen and inhibit nitric oxide formation, to compare said pH to a pH setpoint and activate either said acid solution pump to reduce said pH or said base solution pump to increase said pH, to compare said temperature to a temperature setpoint and to activate either said process cooler to decrease said temperature or said process heater to increase said temperature, to compare said endotoxin concentration to an endotoxin concentration setpoint and activate said endotoxin neutralizing protein solution pump and to compare said dissolved carbon dioxide concentration to a dissolved carbon dioxide concentration setpoint and increase said dissolved carbon dioxide concentration by introducing said stream of carbon dioxide gas into said sparging vessel.

77. (withdrawn) The apparatus of claim 76 wherein said plurality of solution pumps further comprise a proteolytic control means selected from the group consisting of an amino acid solution pump for pumping an amino acid solution from an amino acid solution reservoir into said flow loop and a chelator solution pump for pumping a chelator solution from an chelator

solution reservoir into said flow loop; and wherein said process controller is operative to compare said proteolytic enzyme activity to a proteolytic enzyme activity set point and activate either said amino acid pump or said chelator solution pump.

78. (withdrawn) The apparatus of claim 76 wherein said plurality of solution pumps further comprise a dissolved nitric oxide control means selected from the group consisting of an antibiotic solution pump for pumping an antibiotic solution from an antibiotic solution reservoir into said flow loop and a dextran or heparin solution pump for pumping a dextran solution or a heparin solution from a dextran solution or a heparin solution reservoir into said flow loop; and wherein said process controller is operative to compare said dissolved nitric oxide concentration to a dissolved nitric oxide concentration set point and activate either said antibiotic solution pump or said dextran or heparin solution pump.

79. (withdrawn) The apparatus of claim 76 wherein moving means are provided that are operative to rotate, move linearly or move eccentrically said digestion chamber, valve means are provided that are operative to circulate the physiologic process solution through said digestion chamber in a forward direction and in a reverse direction and transducer means are provided that are operative to cause sonication of the physiologic process solution circulating through said digestion chamber.

80. (previously presented) A method for isolating islets from pancreatic tissue, said method comprising:

filling the reactor of claim 76 with a first portion of said physiologic process solution;

circulating said first portion of said physiologic process solution through said reactor;

draining said first portion of said physiologic process solution from said reactor to produce a rinsed reactor;

refilling said reactor with a second portion of said physiologic process solution by circulating said second portion of said physiologic process solution through said reactor;

pausing the circulation of said second portion of said physiologic process solution through said reactor;

adding the pancreatic tissue to said digestion chamber;

restarting the circulation of said second portion of said physiologic process solution through said reactor and performing real-time data acquisition by said plurality of sensors;

sampling said second portion of said physiologic process solution to determine whether the islets that have been liberated from the pancreatic tissue into said second portion of said physiologic process solution;

when the islets have been liberated from the pancreatic tissue into said second portion of said physiologic process solution, cycling said reactor to affect dilution and collection of the islets.

81. (previously presented) The method of claim 80 wherein circulation of said second portion of said physiologic process solution through said reactor comprises:

flowing said second portion of said physiologic process solution through said reactor in a forward direction; and/or

flowing said second portion of said physiologic process solution through said reactor in a reverse direction.

82. (previously presented) A method for isolating islets from pancreatic tissue, said method comprising:

circulating a physiologic process solution through a reactor having a flow loop that connects a digestion chamber into which said pancreatic tissue has been deposited, a sparging vessel and a process pump, said physiologic process solution having a dissolved oxygen concentration, a dissolved nitric oxide concentration, a pH, a temperature and an endotoxin concentration;

performing real-time data acquisition by means of a plurality of sensors that are exposed to physiologic process solution as it circulates through said flow loop, said plurality of sensors comprising a dissolved oxygen sensor for sensing said dissolved oxygen concentration, a dissolved nitric oxide sensor for sensing said dissolved nitric oxide concentration, a pH sensor for sensing said pH, a temperature sensor for sensing said temperature, and an endotoxin sensor for sensing said endotoxin concentration;

automatically controlling said dissolved oxygen concentration, said dissolved nitric oxide concentration, said pH, said temperature and said endotoxin concentration;

sampling said physiologic process solution to determine whether the islets that have been liberated from the pancreatic tissue into said physiologic process solution;

when the islets have been liberated from the pancreatic tissue into said physiologic process solution, collecting the islets.

83. (withdrawn) An apparatus for isolating islets from pancreatic tissue, said apparatus comprising:

means for circulating a physiologic process solution through a reactor having a flow loop that connects a digestion chamber into which said pancreatic tissue has been deposited, a sparging vessel and a process pump, said physiologic process solution having a dissolved oxygen concentration, a dissolved nitric oxide concentration, a pH, a temperature and an endotoxin concentration;

means for performing real-time data acquisition by means of a plurality of sensors that are exposed to physiologic process solution as it circulates through said flow loop, said plurality of sensors comprising a dissolved oxygen sensor for sensing said dissolved oxygen concentration, a dissolved nitric oxide sensor for sensing said dissolved nitric oxide concentration, a pH sensor for sensing said pH, a temperature sensor for sensing said temperature, and an endotoxin sensor for sensing said endotoxin concentration;

means for automatically controlling said dissolved oxygen concentration, said dissolved nitric oxide concentration, said pH, said temperature and said endotoxin concentration;

means for sampling said physiologic process solution to determine whether the islets that have been liberated from the pancreatic tissue into said physiologic process solution;

means for collecting the islets when the islets have been liberated from the pancreatic tissue into said physiologic process solution.